Food Chemistry **46** (1993) 177–182

Photosensitized decomposition of ascorbic acid in the presence of riboflavin

Ferhunde Şahbaz

Hacettepe University, Food Engineering Department, 06532 Beytepe, Ankara, Turkey

&

Güler Somer*

Gazi University, Department of Chemistry, 06500, Ankara, Turkey

(Received 5 February 1992; revised version received and accepted 2 April 1992)

We studied the riboflavin-sensitized photodecomposition of ascorbic acid by visible light using the polarographic technique. The reaction was carried out at 25°C in the pH range 2·5–6·0 under anaerobic and aerobic conditions and the reaction rate was found to depend on the pH in both cases. Oxygen was consumed during photooxidation and the rate was limited by the riboflavin concentration. Formation of hydrogen peroxide appeared under both aerobic and anaerobic conditions. Reaction steps related to the formation of hydrogen peroxide were included in the reaction mechanisms.

INTRODUCTION

Because the rapid decomposition of ascorbic acid affects food quality directly, its degradation has been a very important subject of research. A number of investigations have been undertaken to determine the factors effective on its decomposition. The main factors of interest include temperature, pH, oxygen concentration, presence of some cations and light (Khan & Martell, 1967*a*, 1967*b*; Ogata *et al.*, 1968; Shtamm & Skurlatov, 1974; Davidson & Grieger-Block, 1977; Sattar *et al.*, 1977; Shtamm *et al.*, 1979). Among these factors, the effect of light is one of the least well known, although the decomposition of ascorbic acid by light has been investigated by some researchers (Sattar *et al.*, 1977; Deschacht & Hendrickx, 1964; Kitagawa, 1968).

The studies that were carried out in orange juice (Granzer, 1983*a*, 1983*b*), in milk (Dunkley *et al.*, 1962) and in infant formula samples (Singh *et al.*, 1976) all showed that ascorbic acid was one of the most light-sensitive vitamins.

Sattar *et al.* (1977) studied the influence of fluorescent light irradiation on ascorbic acid and found that light had no effect on the destruction of the vitamin in pure solutions. The same authors also confirmed the photosensitizing effect of riboflavin on ascorbic acid loss at several concentrations. Similar studies were carried out in solutions containing ascorbic acid and riboflavin, and it was found that the first-order rate constant depended on the pH of the solution (Deschacht & Hendrickx, 1964).

The mechanism of photooxidation of ascorbic acid by flavin mononucleotide has been studied by steady state and flash photolysis techniques (Heelis *et al.*, 1981). The riboflavin-sensitized photooxidation of tryptophan has been studied by Kanner & Fennema (1987). In this research, ascorbic acid was used as an antioxidant but the reaction mechanism was not known.

Light-induced degradation of ascorbic acid in the presence of riboflavin in model systems, however, has not been clearly explained and no reaction mechanism has been suggested which included the formation of hydrogen peroxide. The purpose of this study was to investigate the reaction under different conditions and suggest a mechanism which would fulfil the experimental results.

MATERIALS AND METHODS

Reagents

All the reagents were of analytical reagent grade and obtained from Merck Co., Germany. Thrice distilled water was used for the preparation of the samples and at all other stages of analysis.

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/93/$05.00

Buffer solutions (pH 2·5–6·0)

The pH of the buffer solution was adjusted to the desired value by dropwise addition of 0·5 M KOH to 0·5 M citric acid, using a Knick model pH-meter. Buffer solutions were stable for two weeks.

Sample preparation

This research was performed under aerobic conditions. For the aerobic conditions, a sample solution containing $2{\cdot}27 \times 10^{-3}$ M ascorbic acid and $6{\cdot}4 \times 10^{-7}$–$6{\cdot}4 \times 10^{-5}$ M riboflavin was prepared at the beginning of each run. At the same time, 2·5 ml of citrate buffer was added into the polarographic cell and the temperature was adjusted to 25°C. During this time, the cell was saturated with oxygen by passing air through it for 15 min. Then 2·5 ml of the sample solution prepared as mentioned above was added to the citrate buffer in the cell. In the final solution the ascorbic acid concentration was $1{\cdot}14 \times 10^{-3}$ M and that of riboflavin was $3{\cdot}2 \times 10^{-7}$–$3{\cdot}2 \times 10^{-5}$ M.

When working with an oxygen-free atmosphere, 2·5 ml of buffer, 1·3 ml of glass distilled water and 1 ml of riboflavin solution ($1{\cdot}6 \times 10^{-4}$ M) were put into the polarographic cell and nitrogen was passed through it for 60 min until no oxygen wave was observed in the polarogram. During this time, the cell with thermostat was covered with carbon paper and aluminium foil to protect against daylight. Then 0·2 ml of ascorbic acid solution ($2{\cdot}84 \times 10^{-2}$ M) was added immediately to the reaction cell under nitrogen atmosphere.

Kinetic measurements

The experimental setup mainly consisted of a polarographic cell with thermostat and its auxiliary equipment. The cell was obtained from Princeton Applied Research and fitted with an air-supply system. The flow rate of air or nitrogen (40 ml/min) was adjusted by a flow-meter (Gilmont No. 11) from the Cole–Parmer Company. The stream of air or nitrogen that passed through the cell during the reaction was presaturated with water vapour by streaming it through a wash-bottle.

All the experiments were carried out at 25°C and temperature was controlled by means of a thermostat system (±0·5°C) which contained an immersion circulator from the Cole–Parmer Company (Model 1266-00). The light source was a 100 W tungsten filamented lamp. Especially during illumination, the temperature of the sample solution in the cell was controlled continuously and was determined to be constant all through the experiment. During the experiments without light, the cell was covered with carbon black paper and aluminium foil.

Polarographic measurements

Ascorbic acid

A normal polarographic technique was used to follow the concentration of ascorbic acid during each run. Ascorbic acid has a well-defined anodic current in citrate buffers and the half-wave potential ranged from +170 mV to +20 mV as the pH changed from 2·5 to 6·0.

At the beginning of each experiment, the anodic current of ascorbic acid corresponding to the initial concentration was measured and then anodic waves were recorded in 5 min or 10 min intervals during the reaction. Thus ascorbic acid content was determined by the comparison of wave heights measured at the beginning and during the run.

Riboflavin

The polarographic behavior of riboflavin (1×10^{-5} M) was investigated in the pH range 2·5–6·0 under a nitrogen atmosphere. The cathodic wave of riboflavin with the half-wave potential of −1·32 V vs SCE was detected at pH higher than 6·0, since the supporting electrolyte itself was reduced at this potential when the pH was lower than 6·0.

Oxygen

By measuring the cathodic waves of oxygen, which occurred at about 0 V and −0·8 V in citrate buffers (pH 2·5–6·0), the concentration of oxygen was controlled during the reaction.

Hydrogen peroxide

The formation of hydrogen peroxide as a reaction product was also followed quantitatively (Somer & Green, 1973; Somer & Temizer, 1984) by recording the current of hydrogen peroxide wave with the half-wave potential of about −0·8 V. This wave was the secondary wave of oxygen as mentioned in oxygen determination. For this reason, when working with oxygen-saturated solutions, oxygen present in the solution had to be removed before measurement by the passing of nitrogen in order to find the height of the hydrogen peroxide wave ($E_{1/2} = -0{\cdot}8$ V) formed during the reaction. Under anaerobic conditions, however, hydrogen peroxide could be determined directly. The quantity of hydrogen peroxide was measured by standard addition of hydrogen peroxide solution.

To follow the kinetics of the reaction, the oxidation wave of ascorbic acid was obtained by potential sweep to the positive direction. Then, by potential sweep from 0·0 V to the negative direction, oxygen and hydrogen peroxide were followed.

RESULTS

The following sections will cover the results of the experiments.

Spontaneous oxidation of ascorbic acid

In order to determine the oxidation of ascorbic acid at different pH values, the reaction was followed for 330 min over the pH range 2·5–6·0. The initial rate (r_0) measurements (Table 1, 1st line, riboflavin concentration = 0) showed that ascorbic acid was stable over the

Table 1. $r_0 \times 10^6$ (M/min)[a] for the oxidation and the riboflavin-sensitized decomposition of ascorbic acid[b]

Riboflavin concentration (M)	pH			
	2·5	3·5	4·5	6·0
0·0	0·2 ± 0·1	0·4 ± 0·0	0·5 ± 0·0	4·0 ± 0·1
$3·2 \pm 10^{-7}$	0·3 ± 0·1	1·0 ± 0·2	0·9 ± 0·2	5·4 ± 0·9
$3·2 \pm 10^{-6}$	3·5 ± 0·9	10·6 ± 0·5	11·0 ± 0·8	19·2 ± 2·0
$3·2 \pm 10^{-5}$	9·2 ± 1·5	25·9 ± 0·9	55·6 ± 1·8	92·6 ± 11·9

[a] r_0 = initial rates
[b] T = 25°C; air flow rate = 40 ml/min; initial ascorbic acid concentration = $1·14 \times 10^{-3}$ M; light source = 100 W tungsten filamented lamp.

pH range of 2·5–4·5 but was degraded rapidly at pH 6·0.

The photosensitized decomposition of ascorbic acid in the presence of oxygen and riboflavin

To determine the decomposition of ascorbic acid ($1·14 \times 10^{-3}$ M) by visible light in the presence of $3·2 \times 10^{-5}$ M riboflavin and oxygen, the cell was illuminated, air was passed through and at every 5 min of illumination, the lamp was turned off and the polarogram was taken. The concentration-time plots are shown in Fig. 1 and the initial rates are given in the fourth line of Table 1. Compared to the data for spontaneous oxidation, it was revealed that riboflavin had a significant effect on the light-exposed destruction of ascorbic acid at all values of pH. It was determined that 90% of ascorbic acid was decomposed in 17 min, 21 min and 39 min at the pH values of 6·0, 4·5 and 3·5, respectively.

In order to find whether oxidation losses in ascorbic acid, sensitized by riboflavin, were independent of light, the rate of vitamin decomposition was studied under dark conditions over a period of 30 min. No measureable decomposition was detected for the pH range 2·5–4·5 and only the decomposition rate at pH 6·0 was significant with an initial rate of $3·9 \times 10^{-6}$ M/min. Similar results were also obtained for the decomposition of ascorbic acid when only oxygen was present (Table 1, first line). Therefore it was concluded that light must be present for the sensitizing effect of riboflavin.

Disappearance of the first cathodic wave of oxygen (at 0 V) was also noted in 5 min at pH values of 4·5 or 6·0 when an oxygen saturated solution was illuminated. The amounts of ascorbic acid decomposed over this period at pH values of 4·5 and 6·0 were $2·7 \times 10^{-4}$ M and $3·9 \times 10^{-4}$ M, respectively. Taking into account that the solubility of oxygen in the solution at 25°C is $2·6 \times 10^{-4}$ M, it was concluded that all the oxygen was consumed in the photochemical decomposition process. Since there is a possibility of secondary processes, the initial rates at different pH were measured for comparison (Table 1).

It was found that 10^{-3} M hydrogen peroxide was formed during photodegradation, which was a very important phenomenon never mentioned before. Hydrogen peroxide formed was followed polarographically and its quantity was determined by standard additions of hydrogen peroxide solution.

Effect of riboflavin concentration

The effect of riboflavin concentration on photodecomposition was determined by performing the reaction at $3·2 \times 10^{-7}$ M, $3·2 \times 10^{-6}$ M and $3·2 \times 10^{-5}$ M riboflavin. Inspection of Table 1 shows that the initial rates at each riboflavin concentration increased with increasing pH.

The initial rates of spontaneous oxidation of ascorbic acid, in the pH range 2·5–6·0, were subtracted from the initial rates of photooxidation in the presence of riboflavin. Thus the initial rates of photosensitized oxidation were obtained and plotted against riboflavin concentration (Fig. 2). The results indicated that the rate of photooxidation depended on both the pH and the riboflavin concentration. In the presence of $3·2 \times 10^{-7}$ M riboflavin, the decomposition rate was found to be very low and the effect of pH was not much evident.

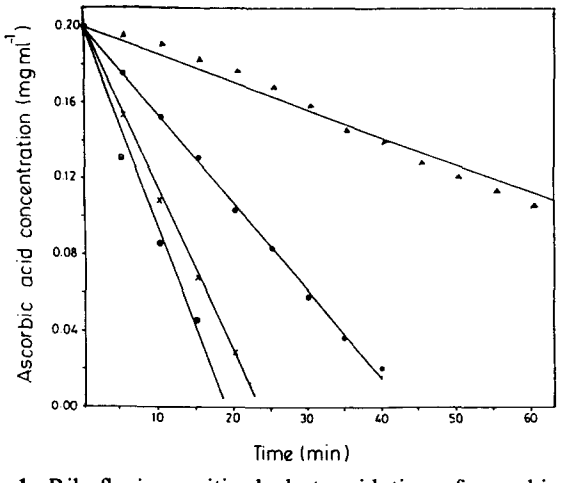

Fig. 1. Riboflavin-sensitized photooxidation of ascorbic acid at the pH values of: (▲) 2·5; (●) 3·5; (×) 4·5; (○) 6·0. Riboflavin concentration = $3·2 \times 10^{-5}$ M; T = 25°C; flow rate of air = 40 ml/min; light source = 100 W tungsten filamented lamp.

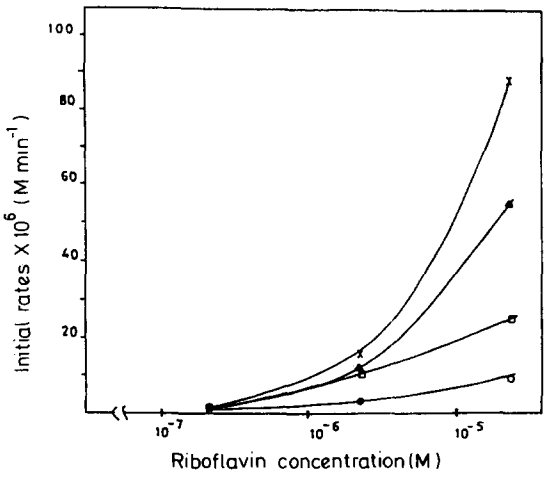

Fig. 2. The initial rates of photosensitized oxidation of ascorbic acid as a function of riboflavin concentration at the pH values of: (○) 2·5; (□) 3·5; (▲) 4·5; (×) 6·0.

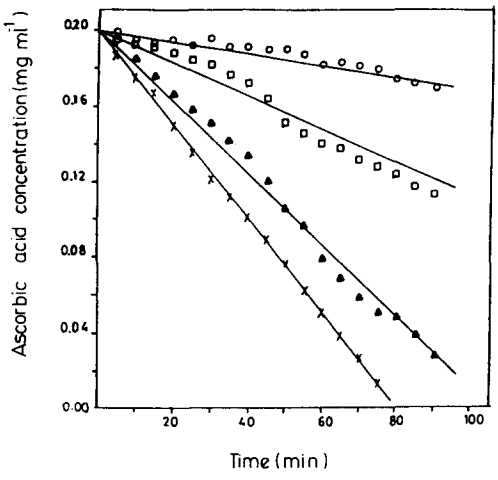

Fig. 3. Photodecomposition of ascorbic acid under anaerobic conditions in the presence of riboflavin at pH values of (○) 2·5; (□) 3·5; (▲) 4·5; (×) 6·0. Riboflavin concentration = $3{\cdot}2 \times 10^{-5}$ M; T = 25°C; flow rate of nitrogen = 40 ml/min; light source = 100 W tungsten filamented lamp.

The photosensitized decomposition of asorbic acid in the presence of riboflavin under anaerobic conditions

The sample solution, ($3{\cdot}2 \times 10^{-5}$ M riboflavin) was illuminated for 80–100 min and the polarograms were taken at 10 min intervals. The concentration versus time plots (Fig. 3) and the initial rates (Table 2) showed that photochemical decomposition proceeded under a nitrogen atmosphere but at rates lower than those observed under aerobic conditions. Also the initial rates increased with increasing pH. It was determined that 90% of ascorbic acid was decomposed in 73 min and 95 min for the pH values of 6·0 and 4·5, respectively.

In these experiments, after de-aerating the solution at the beginning of the experiment, no waves of oxygen at 0 V and −0·8 V were observed. But, after the illumination, a wave appeared at −0·8 V and increased proportionally to the decomposition of ascorbic acid. This was due to the formation of hydrogen peroxide under anaerobic conditions, which was never mentioned by previous workers.

DISCUSSION

The study of the pH-dependence of the rate of photochemical reaction in the pH range 2·5–6·0, both under aerobic and anaerobic conditions, indicated that the rate was dependent on the concentration of the monoascorbate anion, HA^-. When the quantity of monoascorbate ion present at pH 6·0 and the measured initial rate were taken as the base, and the new initial rates were calculated for other pH values, it was found that these calculated rates were smaller than those measured. Therefore, it was concluded that, although the monoascorbate ion was the main species responsible for the photochemical reaction, the neutral form of ascorbic acid may also take part in the reaction.

Table 2. The initial rates for the photodecomposition of ascorbic acid under anaerobic conditions[a]

pH	$r_0 \times 10^6$ (M/min)
2·5	1·8 ± 0·1
3·5	4·3 ± 0·9
4·5	10·2 ± 0·7
6·0	13·2 ± 1·9

[a] Initial ascorbic acid concentration = $1{\cdot}14 \times 10^{-3}$ M; riboflavin concentration = $3{\cdot}2 \times 10^{-5}$ M; nitrogen flow rate = 40 ml/min; light source = 100 W tungsten filamented lamp.

Since ascorbic acid does not absorb in the visible region, it is obvious that riboflavin acts as a photosensitizer. Riboflavin has four absorption peaks at 220 nm, 266 nm, 375 nm and 445 nm. The last two peaks are due to the π—π^* absorption; it has a fluorescence emission at 530 nm (Weimar & Neims, 1975). Irradiation of riboflavin leads to formation of both lumichrome and lumiflavin. Both forms are strong oxidising agents and can catalyze destruction of ascorbic acid.

Mechanism for anaerobic photosensitized decomposition

On the basis of experimental results of the above reactions, a mechanism related to the anaerobic conditions can be proposed:

$$(\text{Rib} \ldots \text{HA}^-) + h\nu \rightarrow (\text{Rib} \ldots \text{HA}^-)^1 \quad (1)$$

$$(\text{Rib} \ldots \text{HA}^-)^1 \rightarrow (\text{Rib} \ldots \text{HA}^-)^3 \quad (2)$$

$$(\text{Rib} \ldots \text{HA}^-)^3 \rightarrow \text{Rib}' + \text{HA}' \quad (3)$$

$$\text{Rib}' + h\nu \rightarrow \text{Rib}^1 \quad (4)$$

$$\text{Rib}^1 \rightarrow \text{Rib}^3 \quad (5)$$

$$\text{Rib}^3 + H_2A \rightarrow RibH_2 + A \quad (6)$$

$$\text{HA}' + \text{HA}' \rightarrow H_2A + A \quad (7)$$

$$\text{RibH}' + \text{RibH}' \rightarrow \text{Rib} + RibH_2 \quad (8)$$

$$\text{RibH}' + \text{HA}' \rightarrow \text{Rib} + H_2A \quad (9)$$

$$RibH_2 + A \rightarrow \text{Rib} + H_2A \quad (10)$$

$$\text{RibH}' + H_2A \rightarrow \text{Rib} + H_3A' \quad (11)$$

where H_2A stands for ascorbic acid, HA^- for the monodehydroascorbic acid and A for dehydroascorbic acid.

This mechanism contains all of the possible reaction steps except the reaction step in which H_2O_2 is formed. The formation of H_2O_2 was not observed in any previous works. In our experiments neither oxygen nor hydrogen peroxide waves could be observed polarographically before illumination, indicating that the residual oxygen concentration was smaller than 10^{-4} M. Since hydrogen peroxide formed was about 10^{-3} M and its concentration increased with the decomposition of ascorbic acid, it was concluded that hydrogen peroxide was formed without oxygen. In this case the only possibility is that H_2O is oxidized by some species, possibly by Rib^3. However, when a solution of riboflavin was illuminated, no H_2O_2 formation was observed. H_2O_2 was formed only when ascorbic acid was also present in the solution. In this case one more step could be added such as:

$$(\text{Rib} \ldots \text{HA}^-)^3 + 2H_2O \rightarrow 2OH' + RibH_2 + \text{HA}'$$

Flavins are well known to associate with a wide range of compounds (Slifkin, 1971). The formation of a triplet of this kind (Step (2)) was shown by Heelis *et al.* (1981) for FMN and the ascorbic acid molecule. In accordance with our pH-dependent rate experiments, monoascorbate ion should appear in the photoreduction (Steps (1), (2) and (3)). Hemmerich (1976) has suggested that flavin photoreactions proceed mainly by a single step involving two electron processes (Step (6)). However, it has been shown (Heelis *et al.*, 1979) that one electron reactions (Steps (3)) are the major mode of the reactivity. In a later publication Hemmerich says that the mode of reaction (one- or two-electron transfer) strictly depends on the substrate (Traber *et al.*, 1982). Steps (7) and (8) are the disproportionation reactions. The same reactions are observed during the photoreduction of FMN by EDTA (Holmström, 1964) and by ascorbic acid (Heelis *et al.*, 1981). RibH' also undergoes a back reaction (Step (9)) with HA', as suggested by Heelis *et al.* (1981) for FMN and by Gillard & Tollin (1974) for phenols and amino acids as donors. Under anaerobic conditions the reaction rate is lower than under aerobic conditions.

Mechanism for aerobic photosensitized decomposition

Any mechanism proposed for photochemical oxidation of ascorbic acid under aerobic conditions must explain the following experimental results:

(1) the direct consumption of oxygen during the photooxidation,
(2) the formation of hydrogen peroxide,
(3) the increase of decomposition with the decreasing hydrogen ion concentration, and
(4) the fact that the rate was limited by the riboflavin concentration.

Besides the given steps for anaerobic conditions, the formation of singlet oxygen should be considered under aerobic conditions. With an energy transfer from triplet riboflavin to oxygen molecule, a very reactive singlet oxygen may be formed. This singlet oxygen reacts with ascorbic acid and monoascorbate anion, forming hydrogen peroxide and dehydroascorbic acid, A. Riboflavin acts both as a Type I and Type II photosensitizer; thus under aerobic conditions both Type I and Type II processes can yield H_2O_2; Type I via $O_2'^-$ and Type II via O_2 (Buettner *et al.*, 1985).

The reaction mechanism under aerobic conditions can be given as the following:

$$\begin{aligned}
\mathrm{Rib} + h\nu &\rightarrow \mathrm{Rib}^1 \\
\mathrm{Rib}^1 &\rightarrow \mathrm{Rib}^3 \\
\mathrm{Rib}^1 + \mathrm{O}_2^3 &\rightarrow \mathrm{Rib}^3 + \mathrm{O}_2^1 \\
\mathrm{H_2A} + \mathrm{O}_2^1 &\rightarrow \mathrm{A} + \mathrm{H_2O_2} \\
\mathrm{HA}^- + \mathrm{H}^+ + \mathrm{O}_2^1 &\rightarrow \mathrm{A} + \mathrm{H_2O_2}
\end{aligned}$$

Under anaerobic conditions, the reaction rate is low because of the steps (9), (10) and (11). In the presence of oxygen, on the other hand, the photodecomposition of ascorbic acid is very efficient, resulting in a high yield. In this case, the back reactions such as steps (9), (10) and (11) must be unimportant. This can possibly be explained by the efficient scavenging of both RibH' and $RibH_2$ by oxygen, which would prevent the back reactions (9), (10) and (11) and hence result in efficient photodestruction of ascorbic acid.

REFERENCES

Buettner, G. R. & Need, M. J. (1985). Hydrogen peroxide and hydroxyl radical production by hematoporphyrin derivative, ascorbate, and light. *Cancer Letters*, **25**, 297–304.

Davidson, R. S. & Grieger-Block, R. A. (1977). The effect of mass transfer on apparent ascorbic acid autoxidation kinetics. *Am. Inst. of Chem. Eng. J.*, **23**(4), 529–38.

Deschacht, W. & Hendrickx, H. (1964). Photolysis of L-ascorbic acid. Influence of the pH upon the decomposition rate. *Mededel. Landbouwhogeschool Opzokingssta. Staat Gent*, **29**(1), 103–18.

Dunkley, W. L., Franklin, J. D. & Pangborn, R. M. (1962). Effect of flourescent light on flavor, ascorbic acid and riboflavin in milk. *Food Technol.*, **16**, 112–18.

Granzer, R. (1983*a*). Effect of light radiation on orange juice in clear glass bottles filled industrially. *Ind. Obst-Gemueseverwert.*, **68**(7), 263–6.

Granzer, R. (1983*b*). Effect of light rays on ascorbic acid and carotenoids content as well as sensory qualities of orange juice in colorless glass bottles. *Fluess. Obst.*, **50**(8), 358–9.

Gillard, J. M. & Tollin, G. (1974). Effect of complexation of flavin radical with tryptophan on electron transfer rates. Model for flavine–protein interactions. *Biochem. Biophys. Res. Commun.*, **58**(1), 328–36.

Heelis, P. F., Parsons, B. J., Philips, G. O. & McKellar, J. F. (1979). The photoreduction of flavine by amino acids and EDTA. A continuous flash photolysis study. *Photochem. Photobiol.*, **30**, 343–7.

Heelis, P. F., Parsons, B. J., Philips, G. O. & McKellar, J. F. (1981). The flavine sensitized photooxidation of ascorbic acid: a continuous and flash photolysis study. *Photochem. Photobiol.*, **33**, 7–13.

Hemmerich, P. (1976). *Fortschr. Chem. Org. Naturst.*, **33**, 451–527. In the flavin sensitised photooxidation of ascorbic acid: a continuous and flash photolysis study. P. F. Heelis, B. J. Parsons, G. O. Phillips and J. F. McKellar, 1981, *Photochem. Photobiol.*, **33**, 7–13.

Holmström, B. (1964). Flash photoreduction of flavine mononucleotide in neutral solutions. Absolute absorptivity of the semiquinone. *Photochem. Photobiol.*, **3**(2), 97–114.

Kanner, J. D. & Fennema, O. (1987). Photooxidation of tryptophan in the presence of riboflavin. *J. Agric. Food Chem.*, **35**, 71–6.

Khan, M. M. T. & Martell, A. E. (1967*a*). Metal ion and metal chelate oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalysed oxidation. *J. Am. Chem. Soc.*, **89**, 4176–85.

Khan, M. M. T. & Martell, A. E. (1967*b*). Metal ion and metal chelate catalysed oxidation of ascorbic acid by molecular oxygen. II. Cupric and ferric chelate catalysed oxidation. *J. Am. Chem. Soc.*, **89**, 7104–11.

Kitagawa, Y. (1968). Studies on the effect of ultraviolet rays on vitamin C. I. Changes of L-ascorbic acid and dehydroascorbic acid. *Eio To Shokurya* **20**(5), 416–421.

Ogata, Y., Kosugi, Y. and Morimoto, T. (1968). Kinetics of

the cupric salt-catalysed autoxidation of L-ascorbic in aqueous solutions. *Tetrahedron*, **24**, 4057–66.

Sattar, A., deMann, J. M. & Alexander, J. C. (1977). Light-induced degradation of vitamins. *Can. Inst. Food Sci. Technol. J.*, **10**(1), 65–8.

Shtamm, E. V. & Skurlatov, Y. I. (1974). Catalysis of the oxidation of ascorbic acid by copper (II) ions. I. Kinetics of the oxidation of ascorbic acid in the copper (II)–ascorbic acid-molecular oxygen system. *Russ. J. Phys. Chem.*, **48**, 852–5.

Shtamm, E. V., Purmal, A. P. & Skurlatov, Y. I. (1979). Mechanism of catalytic ascorbic acid oxidation system Cu^{2+}-ascorbic acid-O_2. *Int. J. Chem. Kinet.*, **11**(5), 461–94.

Singh, P. R., Heldman, D. R. & Kirk, J. R. (1976). Kinetics of quality degradation: Ascorbic acid oxidation in infant formula during storage. *J. Food Sci.*, **41**, 304–8.

Slifkin, M. A. (1971). *Charge Transfer Interactions of Biomolecules*. Academic Press, New York.

Somer, G. & Green, M. E. (1973). Photoreduction of methylene-blue. *Photochem. Photobiol.* **17**, 179–90.

Somer, G. & Temizer, A. (1984). Photoreduction of thionine by water. *Photochem. Photobiol.*, **40**(5), 575–80.

Traber, R., Kramer, H. E. A. & Hemmerich, P. (1982). One and two electron transfer pathways in the photoreduction of flavin. *Pure Appl. Chem.*, **54**, 1651–66.

Weimar, W. R. & Neims, A. H. (1975). In *Physical and Chemical Properties of Flavins: Riboflavin*, Ed. R. S. Rivlin. Plenum Press, New York, pp. 1–47.